



Fruiting bodies of selected edible mushrooms as a potential source of lovastatin

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Abstract

Agaricus bisporus, *Cantharellus cibarius*, *Imleria badia*, and *Lentinula edodes* are among the most popular species of edible mushrooms in Poland. These edible mushrooms are an important source of biologically active substances exhibiting beneficial (e.g., antioxidant, antitumor, antimicrobial, anti-inflammatory) effects on the human body. The fruiting bodies of edible mushrooms are also a valuable source of lovastatin, which belongs to a group of compounds, called statins, commonly used as cholesterol-lowering drugs. Due to the presence of lovastatin, edible mushrooms can be useful in the prevention of hypercholesterolemia. Therefore, the aim of this study was to determine the content of lovastatin in the selected species of edible mushrooms and to evaluate its release into artificial digestive juices. This study was the first to determine the release of lovastatin into digestive juices after the extraction of materials obtained from edible mushrooms. The largest amount of lovastatin was found in the fruiting bodies of *C. cibarius* (67.89 mg/100 g d.w.), and the smallest in those of *L. edodes* (0.95 mg/100 g d.w.). The amount of lovastatin released from the extracts of the examined species into digestive juices was found to be relatively low. The highest content after incubation in artificial gastric juice was detected for the fruiting bodies of *L. edodes* (0.02 mg/100 g d.w.) and after incubation in the intestinal juice for the mycelium from the in vitro cultures of *L. edodes* (0.51 mg/100 g d.w.). Thus, the results of the present study showed that due to its ability to accumulate lovastatin from culture medium, *L. edodes* mycelium can be used to obtain a product with increased hypolipidemic activity.

Keywords Lovastatin · Edible mushrooms · *Lentinula edodes* · Artificial digestive juice · Mycelial culture

Introduction

The interest in edible mushrooms has been constantly growing since the last decade due to their prohealth and therapeutic activities which are proved in many scientific experiments. Edible mushrooms are a source of valuable biologically active substances, including phenolic and indolic compounds, carotenoids, flavonoids, sesquiterpenoids, glucans, glycoproteins, triterpenoids, sterols, tocopherols, antibiotics, vitamins, and bioelements, which are present in them in significant quantities and often act synergistically. It has been proven that these substances exert

immunostimulatory, anticancer, antibacterial, antifungal, anti-inflammatory, and antioxidant effects [1–7]. In addition to these therapeutic compounds, mushrooms contain numerous dietary components and exogenous amino acids. Moreover, the protein derived from the fruiting bodies of mushrooms is well assimilable. The high nutritional value of mushrooms is also associated with their presence of vitamins, minerals, unsaturated fatty acids, and dietary fiber [8–11]. Furthermore, the fruiting bodies of edible mushrooms are a valuable source of lovastatin which belongs to a group of compounds called statins, commonly used as cholesterol-lowering drugs [12–16]. Lovastatin is a naturally occurring compound that selectively blocks the synthesis of endogenous cholesterol. It was first isolated from *Aspergillus terreus* in 1978 and introduced into the medical field by Merck in 1987 [17].

Lovastatin consists of a six-part lactone ring with a hydroxyl group and a partially hydrogenated naphthalene with a hydroxyl substituent esterified with a 2-methylbutyric acid residue (Fig. 1).

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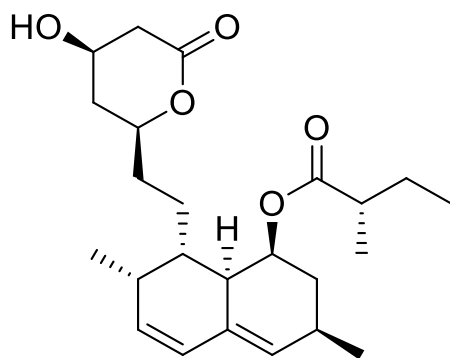


Fig. 1 Structural formula of lovastatin

Lovastatin is present in the living organisms in the form of lactone which is insoluble in water, while its active form β -hydroxy acid is water soluble. Thus, lovastatin is a precursor, which during enzymatic reactions is transformed from the form of lactone to hydroxy acid that competitively blocks 3-hydroxy 3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. As a result, the transformation of HMG-CoA to mevalonate, which is a key stage in the synthesis of cholesterol, is blocked. This mechanism leads to a reduction in cholesterol synthesis, and hence its concentration in the liver, and increases the expression of the low-density lipoprotein receptor on the hepatocyte cell membrane, which is captured from the blood, and decreases its plasma level [17, 18]. Medicines from the statins group also show pleiotropic effects, which include protection of the vascular endothelium and stimulation of its regeneration, stabilization of the atherosclerotic plaque, influence on the clotting system, and involvement in anti-inflammatory and antioxidation processes [19]. It has now been stated that the pathophysiology of atherosclerosis is associated with the induction of inflammatory processes in the vascular wall and, therefore, statins play an important role in the prevention and treatment of this disease.

It has been shown that lovastatin can exert a multidirectional effect in various diseases such as osteoporosis, neurodegenerative diseases, rheumatoid arthritis, ischemic heart disease, and nonalcoholic fatty liver disease [20–24]. Lovastatin also exhibits promising anticancer properties by suppressing the genes involved in cell division, increasing the activity of cell cycle inhibitors and decreasing the activity of cyclins B and D1, activating caspase-3, and inhibiting antiapoptotic factors [25–27]. Lovastatin helps to prevent metastasis and, therefore, it is administered together with anticancer drugs and during radio- and chemotherapies. For instance, this compound can be used in the treatment of malignant mesothelioma since it plays a role in the prevention of cancer proliferation and angiogenesis by inhibiting the activation of vascular endothelial

growth factor-2 (VEGFR-2). Thus, lovastatin, in combination with a VEGFR-2 inhibitor, can exhibit a stronger inhibitory effect on tumor growth [28]. In addition, the antifungal effect of lovastatin has been demonstrated by the growth inhibition of *Rhodotorula rubra*, *Mucor racemosus*, and *Candida albicans* [29].

Due to the presence of dietary substances, edible mushrooms are classified as functional foods [9, 11]. Due to this advantage, it is possible to enrich the mycelium from in vitro cultures with appropriate precursors of active substances to obtain an increased amount of those substances. Various crop modifications are also applied; for example, in the case of *Agaricus bisporus* species, exposing the developing fruiting bodies to UV light leads to a high content of vitamin D₃ [30]. Similarly, lovastatin content can be increased in the fruiting bodies of edible mushrooms by modifying the medium composition and optimizing the culture conditions. Mushrooms also have the ability to accumulate drugs and, hence, there is a possibility to maximize the content of desired drugs in their fruiting bodies [31–33].

The aim of this study was to determine the content of lovastatin in the lyophilized fruiting bodies of four edible mushroom species, namely *A. bisporus*, *Cantharellus cibarius*, *Imleria badia*, and *Lentinula edodes*, and in the lyophilized mycelium from the in vitro cultures of these species. Qualitative and quantitative analyses were performed using reversed-phase high-performance liquid chromatography (RP-HPLC). To determine the actual amount of lovastatin available to the human body, the release of this compound from mushroom material (fruiting bodies and mycelium from in vitro cultures) into artificial digestive juices was evaluated in a Gastroel-2014 apparatus. Thus far, there are no studies available evaluating the release of lovastatin from fruiting bodies and mycelium from the in vitro cultures of edible mushrooms into digestive juices. The present study is the first to determine the content of lovastatin in digestive juices after extraction of the fruiting bodies of edible mushrooms and mycelium from in vitro cultures. This process worked most efficiently for *L. edodes* and, therefore, this species was selected for further research, in which the ability of the in vitro cultures of this species to accumulate lovastatin from the culture medium was evaluated. For this purpose, the medium was enriched with lovastatin and tablets of two commercial preparations containing this compound—Liprox and Lovasterol. The results showed that bioaccumulation of lovastatin by mycelium may allow enriching the mushroom culture to obtain a product with increased anticholesterol activity. In the next stage of the study, the release of lovastatin from the lyophilized biomass of the in vitro cultures of *L. edodes* into artificial digestive juices was evaluated.

Materials and methods

Mushroom material

The fruiting bodies of *A. bisporus* (J.E. Lange) Imbach, *L. edodes* (Berk.) Pegler of commercial origin, and *C. cibarius* Fr. and *I. badia* (Fr.) Vizzini collected from the natural environment—mixed forests of Southern Poland near Nowy Sącz—were used in the experiment. They were cleaned of plants and remnants and then lyophilized. The taxonomic identification was performed with MycoKey 4.1 software (<http://www.mycoskey.com>) by an expert B. Muszyńska. Representative voucher specimens were deposited at the Department of Pharmaceutical Botany, Jagiellonian University Medical College, Krakow, Poland.

Mycelial cultures

To carry out the experiment, in vitro cultures of the above-mentioned species were established on a modified liquid medium according to Oddoux [34].

The mycelial cultures were derived from the previously prepared explants of the fruiting bodies of *A. bisporus* and *L. edodes* of commercial origin as well as from *C. cibarius* and *I. badia* collected from the natural environment. For this purpose, a part of the hymenium was first defatted with 70% ethanol for several seconds, and the material was sterilized with a 15% solution of sodium chlorate for 2 min. Then the fragments of fruiting bodies were rinsed several times with redistilled water and transferred to the Oddoux solid medium (laminar airflow). After 2 weeks of in vitro culture, 250 mL of the modified liquid medium (Oddoux) was transferred to the solid medium.

In the next stage of the experiment, mycelium from the in vitro cultures of *L. edodes* was transferred to 250 mL of the modified liquid medium containing 20 mg of lovastatin in the form of a chemical compound (Sigma-Aldrich, Darmstadt, Germany), weighed on the analytical scales, and in the form of tablets of Liprox and Lovasterol preparations containing 20 mg of lovastatin, respectively.

Experimental cultures

The cultures were established on the liquid medium to obtain the highest possible amount of biomass for use in further analyses. The cultures were shaken at 140 rpm (Altel, Łódź) and then incubated at a temperature of 25 ± 2 °C under a cycle resembling natural light conditions. Liquid in vitro cultures were shaken for 2 weeks, and then again transferred to a new medium to obtain a sufficient amount of biomass for the study.

After 2 weeks of establishing the in vitro culture of the tested mushroom species on the liquid medium, biomass was separated from the medium and rinsed several times with four-time distilled water. The obtained biomass was frozen and then lyophilized. The lyophilized samples were weighed.

Preparation of artificial digestive juices

A solution of artificial saliva with a pH of 6.7 (4.355 g K_2HPO_4 , 3.407 g Na_2HPO_4 , 15.018 g $KHCO_3$, 5.844 g NaCl, 0.142 g $MgCl_2$, 80.114 g citric acid, 1.665 g $CaCl_2$) [35] was prepared by dissolving the weighed contents in a small amount of four-time distilled water. Then the solution was transferred to a flask and topped up with water up to 1000 mL.

A solution of artificial gastric juice with a pH of 2 (2.0 g NaCl and 3.2 g pepsin) [36] was prepared by dissolving the weighed contents in a small amount of four-time distilled water. The solution was transferred to a flask, and then 80 mL of 1 M HCl was added. The solution was topped up with water up to 1000 mL.

A solution of artificial intestinal juice with a pH of 8.0 (0.020 g pancreatic extract, 0.125 g bile salts, and 8.4 g $NaHCO_3$) [37] was prepared by dissolving the weighed contents in four-time distilled water and made up to 1000 mL with water.

Digestion of lyophilized samples of fruiting bodies and biomass from in vitro cultures in artificial digestive juices

An amount of 2.0 g of lyophilized fruiting bodies of *A. bisporus*, *C. cibarius*, *I. badia*, and *L. edodes* and their biomass was weighed. The contents were crushed and then transferred to flasks containing 3 mL of artificial saliva solution. The samples were incubated in this solution for 1 min (approximate physiological time of food residence in the oral cavity).

Then 20 mL of artificial gastric juice was added to each flask. The samples were extracted in this solution at 37 °C for 60 min in a Gastroel-2014 apparatus. The solutions were filtered through a tissue filter, and 20 mL of artificial intestinal juice was added to the biomass obtained. The solutions were incubated under similar conditions for 150 min, and were filtered through tissue filters and then through membrane filters (Millex, Millipore Corporation, USA). The amount of lovastatin in the obtained filtrates was determined using the RP-HPLC method.

Digestion of *L. edodes* mycelium on lovastatin-enriched media in artificial digestive juices

To carry out an experiment on media enriched with lovastatin, lyophilized biomass from in vitro cultures (enriched with lovastatin, Liprox, or Lovasterol) and the control sample were ground separately in a mortar. Then 2.0 g of the test portions was weighed and transferred to flasks. The contents of each flask were moistened with 3 mL of artificial saliva and left for 1 min. Then the contents were decanted and 20 mL of gastric juice was added to each flask. In the next step, they were placed in the Gastroel-2014 apparatus, and the thermostat was set at 37 °C. Each sample was digested in the gastric juice for 60 min. After an appropriate period of time, the contents of the flasks were filtered through pleated filter papers. The filtrate was left to determine lovastatin, and the digested materials together with the filter papers were placed in the flasks. Afterward, 20 mL of artificial intestinal juice was added for further digestion. The flasks containing the prepared samples were placed in the Gastroel-2014 device for 150 min each.

Preparation of methanol extracts

An amount of 2.0 g of lyophilized fruiting bodies of *A. bisporus*, *C. cibarius*, *I. badia*, and *L. edodes* and biomass from the in vitro cultures of these species was weighed. The contents were transferred to beakers containing 100 mL of methanol each. The samples were extracted using ultrasound (Polsonic Sonic 3, frequency 40 kHz) for 20 min, and then the solutions were filtered into crystallizers. The obtained filtrate was evaporated and used for qualitative and quantitative determinations of lovastatin content using the RP-HPLC method.

Determination of lovastatin content in mushroom material using the RP-HPLC method

Chromatographic separation was performed using a high-performance liquid chromatography (HPLC) analyzer (Merck Hitachi). The process was carried out in an isocratic system with a mobile phase of constant composition. The apparatus was equipped with a UV detector ($\lambda = 238$ nm), a column (Purospher RP18 14 × 200 mm, 5 μ m), and a lamp (L7100). During each measurement, 20 μ L of the analyzed sample was injected, and the measurement was performed within 15 min. All measurements were carried out using a previously prepared developing system [acetonitrile and 0.1% phosphoric acid in the ratio of 60:40 (v/v)]. The retention time of the standard substance was 12.75 min.

Extracts obtained after the digestion of fruiting bodies and mycelium from the in vitro cultures of the tested

mushroom species in artificial digestive juices were placed in crystallizers for evaporation. The dry residue was then dissolved in 1.5 mL of methanol and transferred to Eppendorf tubes using membrane filters (Millex, Millipore Corporation, USA). Extracts obtained after extraction using ultrasound and after evaporation were also dissolved in methanol, purified with membrane filters, and then analyzed.

The identification of the lovastatin was made by comparing the retention times of sample peaks with the standard compound. To confirm the presence of lovastatin in the tested extracts, the standard solutions of lovastatin was added as internal standards. The presence of the tested compound in the sample showed up as an increase in peak height for the appropriate retention time. The quantitative analysis of lovastatin was carried out using the calibration curve method. The chromatogram of lovastatin standard is presented in Fig. 2.

Statistical analysis

Statistical analyses were conducted using Microsoft Excel 2010 and the commercially available package GraphPad Prism v3.02 (GraphPad Software, San Diego, CA). The lovastatin concentrations in in vitro cultures of *L. edodes* were evaluated using one-way ANOVA test with Tukey's post hoc multiple comparisons. A p value < 0.05 was accepted as statistically significant. Data were presented as the mean \pm SD from three independent experiments ($n = 3$).

Results and discussion

Determination of lovastatin content in the tested fruiting bodies and in vitro cultures

The least squares linear regression was employed to statistically evaluate the changes in peak areas vs concentration of lovastatin. The calibration was made by the analysis of five standard solutions containing different concentrations of lovastatin in the range 0.0625–1 mg/mL. The linear regression equation was $y = 68756616x + 636261$. The determination coefficient (R^2) obtained for the linear model was found to be 0.9997. The LOD and LOQ values were 0.0144 mg/mL and 0.0435 mg/mL, respectively.

The analysis of the obtained methanol extracts confirmed the presence of lovastatin in the tested species of edible mushrooms. The use of HPLC allowed determining the amount of lovastatin in the methanol extracts, which were obtained from the fruiting bodies of *A. bisporus*, *C. cibarius*, *I. badia*, and *L. edodes* as well as from the in vitro mycelial cultures of these species, and artificial digestive juices. After ultrasound extraction, lovastatin was determined both qualitatively and quantitatively in all the edible

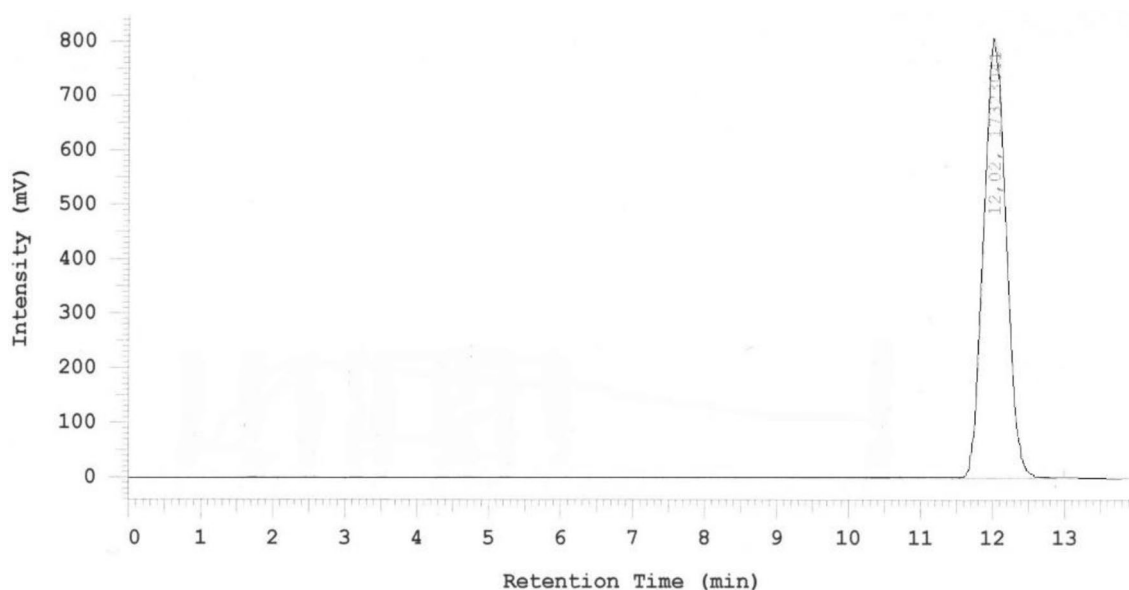


Fig. 2 Chromatogram of lovastatin standard

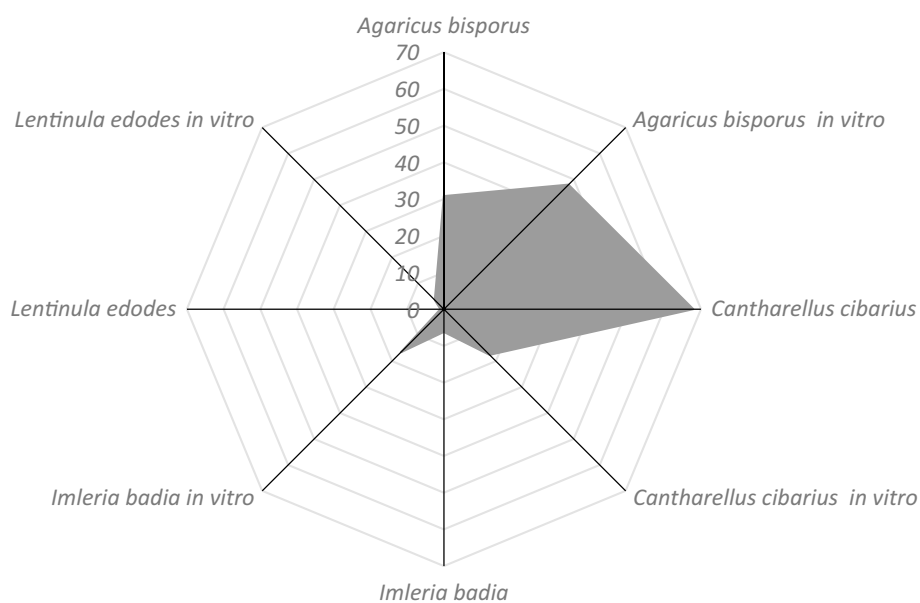
mushroom species investigated. The results of the analyses were expressed as the amount of lovastatin contained in 100 g of dry matter of each of the examined species. The amount of lovastatin in the methanol extracts obtained from the fruiting bodies of the studied species was estimated as follows: *A. bisporus*—30.79 mg/100 g d.w., *L. edodes*—0.95 mg/100 g d.w., *C. cibarius*—67.89 mg/100 g d.w., and *I. badia*—6.21 mg/100 g d.w. Similarly, the amount of lovastatin in the methanol extracts obtained from the in vitro mycelial cultures of the studied species was estimated as follows: *A. bisporus*—47.96 mg/100 g d.w., *C.*

cibarius—17.58 mg/100 g d.w., *I. badia*—16.57 mg/100 g d.w., and *L. edodes*—3.48 mg/100 g d.w. (Fig. 3).

The highest content of lovastatin was found in the fruiting bodies of *C. cibarius* species (67.89 mg/100 g d.w.) and the lowest in the fruiting bodies of *L. edodes* (0.95 mg/100 g d.w.). An exemplary chromatogram for the determination of lovastatin in the methanol extract obtained from the fruiting bodies of *C. cibarius* is shown in Fig. 4.

A study by Chen showed that the fruiting bodies of many edible mushrooms contain significant amounts of lovastatin: *A. bisporus*—56.54 mg/100 g d.w., *Boletus*

Fig. 3 Lovastatin content (mg/100 g d.w.) in the lyophilized biomass of the mushroom species studied obtained after methanol extraction



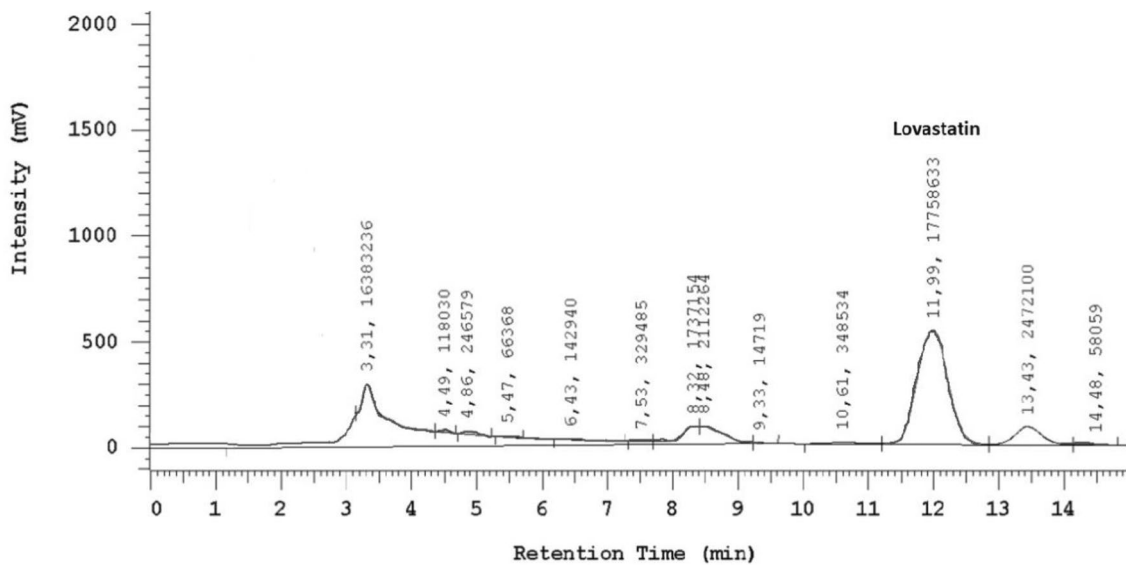


Fig. 4 Chromatogram obtained by analyzing a sample from the fruiting bodies of *C. cibarius*

edulis—32.73 mg/100 g d.w., and *Pleurotus ostreatus*—60.65 mg/100 g d.w. [38]. The study also showed the amount of lovastatin in the fruiting bodies of edible mushrooms collected from different countries: Japan—16.53 mg/100 g d.w., Korea—21.64 mg/100 g d.w., and Taiwan—21.64 mg/100 g d.w. [38]. Lin et al. determined the amount of lovastatin in the fruiting bodies of *L. edodes* as 0.27 mg/100 g d.w. [30]. To date, *P. ostreatus* is most studied for lovastatin content because it is one of the edible mushroom species containing a significant amount of this compound. *P. ostreatus* exerts a hypocholesterolemic effect and was found to inhibit lipid peroxidation in rats and rabbits. It was demonstrated that a diet containing 10% of the dried fruiting bodies of *P. ostreatus* reduces the occurrence and size of atherosclerotic plaques [39]. To our knowledge, no study has determined the lovastatin content in the extracts from the fruiting bodies of *C. cibarius* and *I. badia*.

In the present study, a higher content of lovastatin was found in the extracts from in vitro mycelial cultures compared to the extracts from the fruiting bodies of mushroom species collected from the natural environment, except *C. cibarius*.

In another experiment, the content of lovastatin was determined in two types of in vitro cultures of *P. ostreatus* species (solid-state fermentation—0.33 mg/mL, in-depth fermentation—0.74 mg/mL) and their powdered fruiting bodies (0.47 mg/mL) [13]. Lin et al. determined the content of lovastatin in mycelium from the in vitro cultures of this species to be 2.22 mg/100 g d.w. [30].

Determination of lovastatin content after digestion of the test material in artificial digestive juice solutions

The analysis of the obtained methanol extracts confirmed the presence of lovastatin in the tested mushroom species after extraction into artificial digestive juices. The results of the analysis were expressed as the amount of lovastatin released from 100 g of dry matter of each of the tested species. Both artificial gastric juice, after 60-min incubation preceded by incubation of the material in artificial saliva, and artificial intestinal juice, after 150-min incubation of the tested material preceded by incubation in artificial saliva and artificial gastric juice, were tested. An example of a chromatogram of lovastatin extracted from artificial digestive juices is shown in Fig. 5.

After incubation of the examined material in artificial gastric juice, lovastatin was determined qualitatively and quantitatively only for *L. edodes* (0.02 mg/100 g d.w.). In the samples of the other species, the content of lovastatin was less than 0.01 mg/100 g d.w.

The release of lovastatin into artificial intestinal juice after 150 min of incubation (after a previous incubation in artificial saliva solution and artificial gastric juice) was confirmed in the samples of all the tested species of edible mushrooms, except the *A. bisporus* mycelium obtained from in vitro cultures; however, in most samples, the amount of lovastatin was below 0.01 mg/100 g d.w. The highest content of lovastatin was found in the extract from the in vitro cultures of *L. edodes* (0.51 mg/100 g d.w.). Figure 6 shows

Fig. 5 Example of a chromatogram obtained by the analysis of *L. edodes* extract from in vitro cultures on modified Oddoux medium after 60-min incubation in gastric juice and then 150-min incubation in intestinal juice

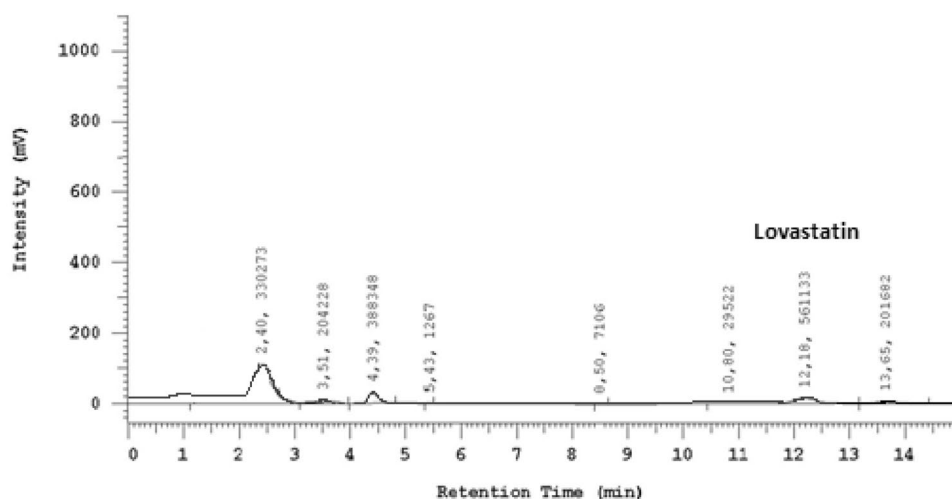
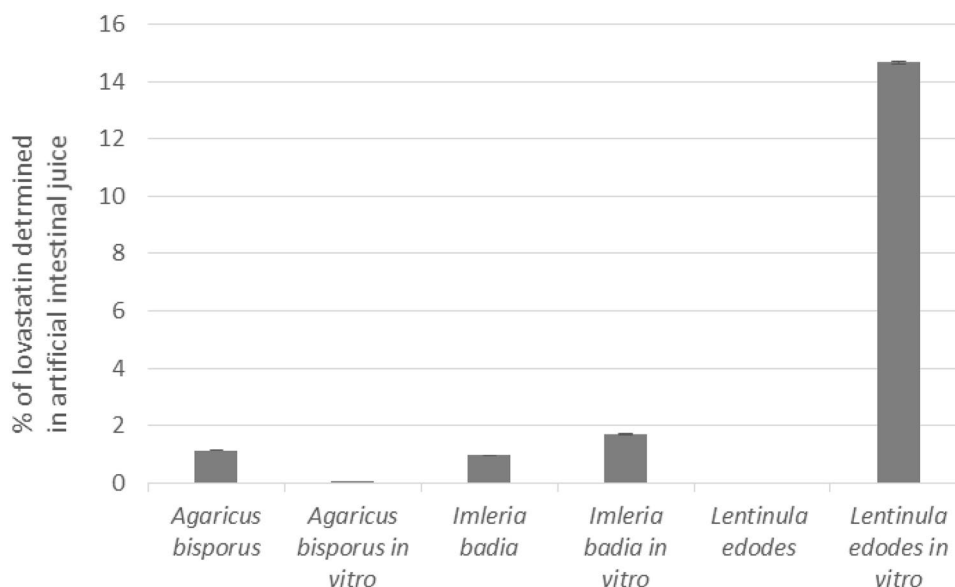


Fig. 6 Percentage of lovastatin determined in artificial intestinal juice in relation to its total content in the methanol extract of the mushroom materials tested



the percentage of lovastatin determined in artificial intestinal juice in relation to the total content determined in the methanol extract of the mushroom materials studied. Because the in vitro cultures of *L. edodes* were found to have the highest content, this species was selected for further study.

Evaluation of *L. edodes* ability to accumulate lovastatin

The experiment showed that mycelium from the in vitro cultures of *L. edodes* established on a modified Oddoux medium enriched with lovastatin yielded a higher biomass than the control sample cultured without the addition of lovastatin. Biomass gains were as follows: 3.22 g/250 mL medium for *L. edodes* + lovastatin, 1.65 g/250 mL medium for *L. edodes* + Liprox, 1.62 g/250 mL medium for *L. edodes* + Lovasterol, and 0.83 g/250 mL medium for *L.*

edodes (control sample). Although the highest amount of biomass was obtained from the in vitro cultures enriched with lovastatin, enrichment with commercial preparations also resulted in a significantly higher amount of biomass compared to the control sample. From the biotechnological point of view, this means that it is possible to obtain in vitro cultures of *L. edodes* with an increased hypocholesterolemic activity.

The analysis of the results showed that lovastatin is present in each of the studied species of edible mushrooms, but it is released into artificial digestive juices only in very small amounts. The highest content after incubation in artificial gastric juice was detected in the fruiting bodies of *L. edodes* (0.02 mg/100 g d.w.) and after incubation in the intestinal juice in mycelium from the in vitro cultures of *L. edodes* (0.51 mg/100 g d.w.).

To evaluate the ability of *L. edodes* mycelium to accumulate lovastatin, the content of this substance was determined in the mycelium from the in vitro cultures of this species established on Oddoux medium enriched with 20 mg of lovastatin per 250 mL of medium or one tablet of Liprox or Lovasterol (20 mg of lovastatin) per 250 mL of medium. It was demonstrated that the in vitro cultures of *L. edodes* are capable of accumulating lovastatin. Its content in mycelium from the lovastatin-enriched medium was significantly higher compared to the control sample. The in vitro cultures on nonenriched medium contained 3.48 mg of lovastatin per 100 g of dry matter, while those grown on enriched medium contained up to 100 times more lovastatin per 100 g of dry matter. Thus, significant differences ($p < 0.05$) were found in the content of lovastatin between the control *L. edodes* mycelium and the mycelium grown on Oddoux medium enriched with this substance or with one tablet of Liprox or Lovasterol. The most effective bioaccumulation of lovastatin was observed in the in vitro cultures enriched with pure substance compared to the two commercial preparations (Fig. 7).

Examination of lovastatin release from mycelium from the in vitro cultures of *L. edodes* into artificial digestive juices

It was shown that lovastatin from biomass obtained from the in vitro cultures of *L. edodes* on medium not enriched with the tested substance did not release into artificial digestive juices. Both in gastric and intestinal juice, lovastatin was found in amounts not exceeding the limit of quantification. In the case of in vitro cultures of *L. edodes* established on modified Oddoux medium enriched with lovastatin (both preparations and pure compound),

lovastatin was found in intestinal juice after digestion of the tested material for 30 min in gastric juice. The results showed no clear correlation between the amount of lovastatin accumulated in the mycelium from in vitro cultures and the amount of lovastatin released into artificial digestive juices (Fig. 8). Studies on the bioavailability of biologically active substances from mushroom material are scarce. Several reports on the release of bioelements and other organic compounds from food including mushrooms into artificial digestive juices were published, but study of bioavailability of lovastatin from mushroom is a new research direction [40–43]. It is well known that lovastatin is present in edible mushrooms from the Basidiomycota taxon, and their regular consumption can lead to lowering cholesterol levels and thus preventing from cardiovascular diseases [43, 44]. There are, however, reports on the bioavailability of lovastatin present in red yeast rice products [45]. It was proved that this natural material may contain lovastatin with a higher dissolution rate and reduced crystallinity compared to commercially available formulations, making it more bioavailable [45]. In addition, previous experiments on the bioavailability of lovastatin have shown that it is a substance with low bioavailability, which is why it is necessary to create forms of lovastatin that will allow the most effective absorption in the human body [45–47].

On the basis of the conducted analyses, it was found that the content of lovastatin was higher in the in vitro cultures than in the fruiting bodies of the tested mushrooms. This study proved the low availability of lovastatin after consumption, despite its high content in methanol extracts of the abovementioned species. Therefore, to utilize the antiatherosclerotic effect of mushrooms, alcoholic extracts are recommended.

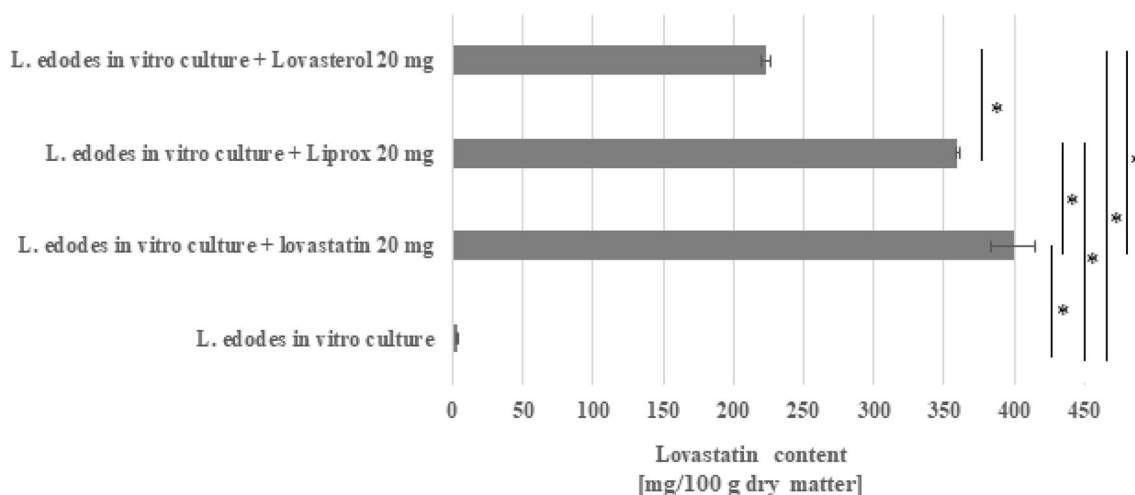
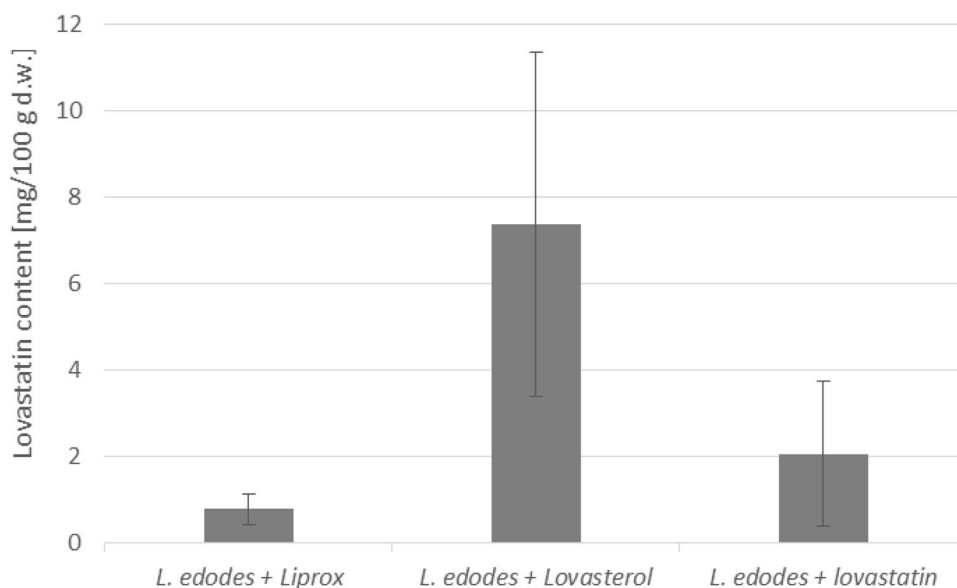


Fig. 7 Lovastatin content in biomass extracts from the in vitro cultures of *L. edodes* (mg/100 g dry matter). The data are presented as a mean SD from three independent experiments ($n = 3$). For statistically significant results, $P < 0.05$

Fig. 8 Lovastatin content extracted into artificial intestinal juice after digestion in gastric juice for 30 min from lyophilized biomass from the in vitro cultures of *L. edodes* (mg/100 g dry matter)



Conclusion

In vitro cultures of *L. edodes* effectively accumulated lovastatin and, therefore, the in vitro culture media can be supplemented with this compound to obtain mycelium with high hypocholesterolemic activity. Additionally, enrichment of the medium with lovastatin increased the biomass gains in relation to the cultures not enriched with this compound. The amount of lovastatin accumulated in mycelium from the medium enriched with commercial preparations was lower than that from the medium enriched with pure compound, but the ability to release the compound into artificial digestive juices differed between the cultures enriched with commercial preparations and pure substance.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human participants or animal performed by any of authors. No ethical approval was required.

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